THIS REVISION CONTAINS CLARIFICATION TO Section 3. DERMAL ABSORPTION.

HED DOC. NO. 014390

DATE: November 30 , 2000

MEMORANDUM

SUBJECT: ***DIAZINON*** **- REEVALUATION OF THE TOXICITY ENDPOINTS SELECTED FOR DERMAL RISK ASSESSMENTS AND SELECTION OF THE ENDPOINTS FOR SHORT TERM AND INTERMEDIATE TERM INCIDENTAL INGESTION -** ***REVISED*** Report of the Hazard Identification Assessment Review Committee.

FROM: John Doherty
Toxicologist
ReRegistration Branch III
Health Effects Division

THROUGH Jess Rowland, Co-Chair
and
Elizabeth Doyle, Co-Chair
Hazard Identification Assessment Review Committee
Health Effects Division (7509C)

TO: Steve Knizner, Branch Senior Scientist
ReRegistration Branch III
Health Effects Division (7509C)

PC Code: 057801

On **October 5, 2000**, the HIARC reevaluated the doses and toxicity endpoints selected for dermal exposure risk assessments at the previous (February/March, 1999) meetings based on the comments received during Phase 3 (Public Comment) of the Tolerance Reassessment Advisory Committee (TRAC) process. In addition, the HIARC selected doses and endpoints for incidental oral ingestion exposure

resulting from residential uses. The Committee's conclusions are presented in this report.

Committee Members in Attendance

Members present were: Ayaad Assad, William Burnam, Jonathan Chen,, Elizabeth Doyle, Pam Hurley, Elizabeth Mendez, Jess Rowland, and Brenda Tarplee

Other HED staff present at the meeting were: John Doherty, Tim Leighton, and Deborah Smegal from HED and Benjamin Chambliss (RD).

I. BACKGROUND

On **February 20, 1997** the Health Effects Division's RfD/Peer Review Committee established a Reference Dose (RfD) of 0.0007 mg/kg/day based on a NOEL of 0.02 mg/kg/day established in a human volunteers and an Uncertainty Factor of 30 which included 10 x for intra-species variability and 3 x to account for the close proximity of the NOEL (0.02 mg/kg/day) and LOEL (0.025 mg/kg/day) established as well as the use of only one sex (males) in the critical study. The NOEL established in the human study was supported by the results observed in animal studies (*Memorandum:* G. Ghali, HED to George LaRocca, RD, dated June 17, 1997).

On **February 25, 1997**, the Health Effects Division's Toxicology Endpoint Selection (TES) Committee selected the doses and endpoints for acute dietary as well as occupational and residential exposure risk assessments (TES Document dated 6/4/97; HED DOC. NO. 013157).

On **March 17, 1998**, the Health Effects Division's Hazard Identification Assessment Review Committee (HIARC) re-evaluated the existing Reference Dose (RfD) and the toxicological endpoints selected for acute dietary and occupational/ residential exposure risk assessments, and determined the Uncertainty Factors and/or Margins of Exposure for the various exposure scenarios. The HIARC also addressed the potential enhanced sensitivity of infants and children from exposure to Diazinon as required by the Food Quality Protection Act (FQPA) of 1996.

On **February 16, 1999** and again on **March 4, 1999**, to comply with OPP's current policy of not using human data in risk assessments, the HIARC selected the doses and toxicology endpoints for all exposure scenarios based solely on animal toxicity studies**.** In addition, the HIARC determined the appropriate uncertainty factors and MOEs (HIARC Reported dated, September 21, 1999; HED Document No. 013745).

On **October 5, 2000**, the HIARC reevaluated the doses and toxicity endpoints selected for dermal exposure risk assessments at the previous (February/March, 1999) meetings based on the comments received during Phase 3 (Public Comment) of the Tolerance Reassessment Advisory Committee (TRAC) process. The Registrant contends that the Agency should not use the default assumption of 100% dermal absorption factor for diazinon to modify the oral dose when performing dermal risk assessments. The HIARC previously selected the 100% dermal absorption value based on the similarity of results seen following oral and dermal administration. The Registrant stated that a weight-of-evidence support a dermal absorption factor of 3.58 % based on an *in vivo* percutaneous absorption study of diazinon in human volunteers and new data from an exposure monitoring study of homeowners applying diazinon products which showed that the dermal absorption of diazinon is 6.1%. The HIARC reviewed these data as well as the 21-day dermal toxicity study in rabbits and the Committee's conclusions are presented in this report.

I. HAZARD IDENTIFICATION

No revisions were made for the doses and endpoints selected for establishing the Acute and Chronic Reference Doses. They are presented here for clarity and transparency only. For details refer to the HIARC Report dated September 21, 1999, HED Document N 013745

A. Acute Dietary Reference Dose (RfD)

Studies selected: Acute Neurotoxicity - Rat
Special study to determine the NOAEL for Cholinesterase Inhibition -Rat

MRID No(s): 43132204 and 44219301

Executive Summary: For acute dietary risk assessment, the HIARC selected the NOAEL of 0.25 mg/kg based on inhibition of plasma cholinesterase activity in female rat observed at 2.5 mg/kg (LOAEL). This NOAEL was established based on the results of two single dose studies in male and female Sprague-Dawley rats.

In the acute neurotoxicity study (MRID No. 43132204), rats (15/sex/dose) received diazinon (D-Z-N technical 88% purity) in corn oil by gavage. at 0, 2.5, 150, 300 or 600 mg/kg. . Clinical signs, FOB and motor activity assessed in 10/sex/dose; the other five were assessed for ChE/AChE activity. Plasma cholinesterase activity (ChE) was inhibited at all dose levels (27% for males and 47% for females in the 2.5 mg/kg dose group) and RBC AChE was inhibited at 150 mg/kg (83% for males and 76% for females) at the time of peak effect (about 9 hours postdosing). ChE was equivalent to the controls at day 15 but RBC AChE still remained inhibited for both males and females especially at the higher dose levels. Brain AChE was unaffected when assessed at day 15. The LOAEL for RBC AChE inhibition was 150 mg/kg. The NOAEL for RBC AChE inhibition was 2.5 mg/kg. The LOAEL for plasma ChE inhibition is < 2.5 mg/kg.

Since a NOAEL for plasma cholinesterase inhibition was not established, another study was conducted.

In that study (MRID No. 44219301),at 0, 0.5, 0.5, 1, 10, 100 or 500 to males and at 0, 0.05, 0.12, 0.25, 2.5, 25 or 250 mg/kg to females and sacrificed ~24 hours later. Observations on their behavior reactions were noted and the blood and brain were assessed for ChE/AChE. The precision of the ChE/AChE assays was considered fair to poor but not sufficiently poor to preclude an assessment of the potential for diazinon to inhibit ChE/AChE. Plasma ChE was inhibited at 2.5 mg/kg in females (61%) and at 10 mg/kg in males (44%). RBC AChE was inhibited at 25 mg/kg in females (35%) and at 100 mg/kg in males (49%). Brain AChE was inhibited at 25 mg/kg in females (36%, not significant) and at 250 mg/kg (70%) and at 500 mg/kg in males (69%). **The LOAEL was 2.5 mg/kg based on**

61% plasma ChE inhibition in females. The NOAEL is 0.25 mg/kg.
Dose and Endpoint Selected for Establishing the Acute RfD = 0.25 mg/kg. based on inhibition of plasma cholinesterase activity in female rat observed at 2.5 mg/kg (LOAEL).

Uncertainty Factor (UF): 100 (10 x for inter-species extrapolation and 10 x for intra-species variability).

$$\textbf{Acute RfD} = \frac{0.25 \text{ mg/kg(NOAEL)}}{100 \text{ (UF)}} = \textbf{0.0025 mg/kg}$$

Comments about Study/Endpoint/Uncertainty Factor: No change from the previous dose/endpoint based on the rat studies (i.e., human data was not used previously). The TES Committee did not use the human study because reproducible animal studies were available following a single exposure as demonstrated in the two studies discussed above.

B. Chronic Dietary RfD

Studies selected: 4 week, 90 day and 1 year feeding studies in dogs and 28 day feeding special cholinesterase study, 90 day neurotoxicity, 90 day feeding and chronic feeding studies in rats.

MRID No(s): See Section VII of the HIARC Report dated September 21, 1999

Dose and Endpoint for establishing the Chronic RfD = 0.02 mg/kg/day based on the consistent pattern of **no** adverse effects on cholinesterase inhibition.

Uncertainty factor = 100 (10 x for inter-species extrapolation and 10 x for intra-species variability).

$$\textbf{Chronic RfD} = \frac{0.02 \text{ mg/kg/day}}{100 \text{ (UF)}} = \textbf{0.0002 mg/kg/day}$$

Comments about Study/Endpoint/Uncertainty Factor: A weight-of-evidence (WOE) approach was used for the dose and endpoint selected for use in the chronic dietary risk assessment. The WOE is based upon an analysis of seven oral repeated dose studies, four in the rat and three in the dog. The results of these studies, taken *in toto,* demonstrated that at 0.02 mg/kg/day a consistent pattern of no adverse effects was achieved. The data for the rat which were considered included: an oral 28-day study, a 90-day feeding study, a 90-day oral neurotoxicity study and a two year feeding study. In the first three studies 0.02 mg/kg was clearly established as a NOAEL based upon statistically significant plasma cholinesterase inhibition at the next higher doses. In the two year feeding study, the dose levels did not include a 0.02mg/kg level, but the lowest two doses, 0.004/0.005 mg/kg in males and females, respectively and 0.06/0.07 in males and females, respectively, bracketed this level. Although at the 0.06 mg/kg level there was statistically significant depression in plasma cholinesterase in females in 4/5 time point measurements, the males (0.07 mg/kg) showed much more variability at this dose and had statistically significant plasma cholinesterase depression only in 1/5 time point measurements. At the lowest dose, 0.004 mg/kg the males exhibited the same variability in plasma cholinesterase

measurement although none of the levels reached statistical significance. Given the fact that there is no consistent pattern of plasma cholinesterase between the sexes, and the 0.06 mg/kg level appears to be a minimal effect level while the 0.004 mg/kg level is clearly a no-effect level, the 0.02 mg/kg level, common to the other three studies, was judged to be an overall NOAEL level for the rat.

The data for the dog which were considered included: a 4-week pilot feeding study, a 90-day feeding study and a one-year feeding study. Each of these studies had a common dose level of 0.02 mg/kg. In each of these studies the only effect seen at that dose level was plasma cholinesterase inhibition. In the 4-week pilot only females had a statistically significant inhibition of plasma cholinesterase which appeared to reach steady state between 14-25 days of dosing. In the 90-day study only males had a statistically significant inhibition of plasma cholinesterase at 0.02 mg/kg and only on days 29 and 86. In this study steady state levels of plasma cholinesterase inhibition were reached between days 30 and 90. In the one year study there were statistically significant decreases in plasma cholinesterase in females in 2/4 time point measurements at the lowest dose of 0.0037, but these decreases were considered not biologically relevant due to the inconsistency across time and the variability of the magnitude of the decreases. At the next dose, 0.02 mg/kg, the only effect observed was statistically significant plasma cholinesterase inhibition in females across all time points and in males only midway in the study at day 176. No other effects were seen in any of the studies at the 0.02 mg/kg dose. The plasma cholinesterase inhibition at 0.02mg/kg is considered to be a minimal or borderline effect in the dog since there were no effects on either the blood or brain cholinesterase levels, and there was no consistent pattern of cholinesterase inhibition between the sexes at this level.

In summary, the chronic dietary endpoint is based upon the results of seven studies in the dog and rat which point to 0.02 mg/kg/day as the appropriate level on which to conduct the chronic dietary risk assessment.

Although 0.02 mg/kg/day was selected based on the results of short and long-term studies, no additional uncertainty factors were deemed necessary since: 1) the principal effect (plasma cholinesterase inhibition) was considered to be minimal or borderline, primarily there were no other effects observed at this dose (e.g., no red blood cell or brain cholinesterase inhibition nor clinical signs of toxicity or systemic effects), and there were no consistent pattern of cholinesterase inhibition between the sexes at this level ; 2) a steady state of plasma cholinesterase inhibition was reached by 30 to 90 days in the dog; and 3) this dose (0.02 mg/kg/day) was a clear NOAEL in rats.

C. Occupational / Residential Exposure

1. Short-Term Incidental Oral Ingestion (1 - 7 days)

Studies selected: Acute Neurotoxicity - Rat
Special study to determine the NOAEL for Cholinesterase Inhibition -Rat
MRID No(s): 43132204 and 44219301

Executive Summary: Refer to Acute RfD

Dose and Endpoint Selected for Risk Assessment: 0.25 mg/kg based on inhibition of plasma cholinesterase activity in female rat observed at 2.5 mg/kg (LOAEL).

Comments about Study/Endpoint: This principal toxicological effect is appropriate for the population (toddlers) and duration (1-7 days) of concern.

2. Intermediate -Term Incidental Oral Ingestion (7 days to Several Months)

Studies selected: 90-day and 1- year feeding study in dogs

MRID No(s): 40815004 and 41920001

Dose selected for Risk Assessment = 0.02 mg/kg/day based on the consistent pattern of no adverse effects on cholinesterase inhibition.

Comments about Study/Endpoint: In the 90-day dog feeding study, no effects were observed in either sex at the lowest dose of 0.0034 mg/kg/day in males and 0.0037 mg/kg/day in females. At the next higher dose of 0.02 mg/kg/day (both sexes), the only effect noted was plasma ChE inhibition reaching statistical significance in males only on days 29 and 86. However, the magnitude of inhibition in males was consistent across time on the days measurements were taken during the study [day 29 (29%), day 56 (27%), day 86 (30%)]. Corresponding values for females (expressed as percent inhibition) ranged from (15 to 17% and were not statistically significant). Examination of the pattern of plasma ChE activity over time indicated that a steady state level of inhibition was reached by 90 days and possibly as early as 30 days (in other words, no considerable increase in plasma cholinesterase inhibition would be expected after 30 to 90 days of continuous dosing). This observation was supported by a similar examination of the blood cholinesterase data in the 1 year study (which also contained a measurement time point at approximately 90 days). This principal toxicological effect is appropriate for the population (toddlers) and duration (7 to 90 days) of concern.

3. Dermal Absorption

In September, 1999, the HIARC determined that the 100% default value (equivalent to oral absorption) is appropriate based on the similarity of results observed following oral and dermal administration. Since then, the Registrant has submitted an *in vivo* dermal absorption study (MRID No. 44982801) in which 6 adult male human volunteers/group received dermal applications of ^{14}C-diazinon as follows: Group A: 2 ：g/cm^2 in the ventral forearm; Group B: 2 ：g/cm^2 on the abdomen; and Group C: 147 ：g/cm^2 on the abdomen. The application site was washed with soap and water after 24 hours and tape stripped after 7 days.. Total urine was collected for 7 days and analyzed for radiolabel. Five rhesus monkeys were dosed intravenously with ^{14}C- diazinon and total urine and feces collected for 7 days. Urine and feces were analyzed for radiolabel. Monkey urinary excretion of radiolabel (56%) was used to correct for human urinary excretion of radiolabel as a measure of

absorbed dose. The following table summarizes the results of this study.

Group/Dose	Application Site	Formulation Vehicle	Skin Wash %	Tape Strip %	Urine %	Total Recovery %*	Absorbed %
A/2 µg/cm^2	Ventral Forearm	Acetone	0.4566	0.0096	1.9983	2.4645	3.5684
B/2 µg/cm^2	Abdomen	Acetone	1.4448	0.0060	1.8095	1.9603	3.2313
C/1.47 µg/cm^2	Abdomen	Lanolin	0.3543	0.0421	1.2757	1.6721	2.2780

*Note: 97 to 98% of the administered dose is not recovered and unaccounted for.

In this study, dermal absorption in humans ranged from 2.28 to 3.57%.

In addition, the Registrant recently (September, 2000, MRID No.: 45184305) submitted data from an exposure monitoring of homeowners mixing and applying readily available liquid products to their lawn. In one phase of the study (passive dosimetry), external exposure (dermal and inhalation) to diazinon was determined. In the second phase of the study (urine biomonitoring), internal exposure to diazinon was based on their urinary excretion of G-27550, a unique urinary metabolite of diazinon. The percent dermal absorption of diazinon determined by comparing the internal absorbed diazinon dose to the external diazinon dermal exposure. The Registrant contends that data from this study showed that the dermal absorption of diazinon is 6.1%. However HED conducted an independent analysis of this study and concluded that dermal absorption was highly variable (range <1 to 58%) depending on the individual techniques and application equipment used. This conclusion was based on comparing the passive dosimetry and biomonitoring exposures for the same individual. Average dermal absorption ranged from 4 to 14% (See Memorandum from D. Smegal to D. Drew/B. Chamblis, dated, November 29, 2000).

The Registrant during the Phase 3 comments contended that the data from the homeowner monitoring study provided independent verification of the 3.58% dermal absorption demonstrated in the in vivo percutaneous absorption study. The weight-of-evidence, therefore, according to the registrant, supports a dermal absorption factor of 3.58% for diazinon, not 100% as previously determined by the HIARC.

The rabbit oral developmental (MRID No.: 00079017) and 21-day dermal (MRID No.: 40660806) toxicity studies which both show deaths at 100 mg/kg/day provided data to show that the 100% (default value) dermal absorption factor may apply to rabbit skin. However, the dermal absorption data from the Wester and Maibach study (MRID No: 44982801) indicate that in humans dermal absorption is very low and this appears to be supported by the biomonitoring study (MRID No.: 45184305) data. There is thus no consistency across species with regard to dermal absorption. The discrepancy between estimating a dermal absorption factor based analysis of diazinon equivalents in the urine as was done in the *in vivo* human study to an estimated dermal absorption factor based on comparison of the rabbit oral and dermal studies that are based on expression of toxicity (deaths) may reflect the sum of the differences in the

pharmacokinetics and metabolism of diazinon in each species as well as the susceptibility of the rabbit to diazinon toxicity once absorbed. Partly because of the inconsistency in data available to estimate a dermal absorption factor, the HIARC determined that a dermal absorption factor will not be used for risk assessments for dermal exposure. The HIARC selected the route-specific 21-day dermal toxicity study with rabbits because the use of a dermal toxicity study eliminates the uncertainty associated with the selection of a dermal absorption factor for route to route extrapolation.

4. Short-Term Dermal (1-7 Days)

Study Selected: 21-day Dermal Toxicity - Rabbit

MRID No: 40660807

Executive Summary: Note: As prepared by J. Doherty following deliberations at the October 5, 2000 meeting of the HIARC.

In a 21-day dermal toxicity study in rabbits (MRID No.: 40660807), four groups of New Zealand strain rabbits (5/sex/dose) initially were dosed as control, 1, 5 or 100 mg/kg/day of diazinon (97.1% suspended in 50% polyethylene glycol). The rabbits were dosed 5 consecutive days/week for three weeks (15 applications) and the test material remained in contact for 6 hours.
The original high dose group was excessively toxic since 4 of the 5 males died following tremors and other signs of cholinergic reactions on days three to six. The high dose was then reduced to 50 mg/kg/day. Hematology and clinical chemistry were assessed at termination. Serum cholinesterase and RBC and brain acetylcholinesterase was assessed by diagnostic kit (Berringer Mannheim Diagnostics).

There were some indications of increased weight gain and food consumption in the rabbits dosed all doses of diazinon but there was no dose response and it considered that the data were too few animals on the study to make a more definite evaluation. **The LOAEL for systemic toxicity is 100 mg/kg/day based on deaths associated with cholinergic inhibition symptoms. The NOAEL is 50 mg/kg/day.**

Serum ChE in females demonstrated 32% ($p < 0.05$), 35% ($p< 0.01$) and 62% ($p < 0.01$) inhibition for the 1, 5 and 50 mg/kg/day dose groups respectively relative to the control group based on group means after three weeks. When compared to the predosing baseline, this progression was 16%, 18% and 57% ($p < 0.01$). Thus, there was no dose response between the 1 and 5 mg/kg/day dose groups. Statistical evaluation by HED staff using pair-wise analysis indicated that only the mid and high dose groups were statistically significant although a trend was evident for all groups. For males, statistically significant inhibition of plasma ChE was evident at 50 mg/kg/day only (64% $p < 0.05$) although there was 23% apparent inhibition at 5 mg/kg/day. **RBC AChE** was statistically significantly decreased at 50 mg/kg/day (39% for males and 32% for females, both $p < 0.01$). **Brain AChE** in females was decreased at 5 mg/kg/day (18% $p < 0.05$) and 50 mg/kg/day (43% $p < 0.05$. In males

there was only one surviving rabbit and brain AChE was decreased 28%. **The LOAEL for inhibition of serum ChE and brain AChE is 5 mg/kg/day based on data in females. The NOAEL is 1 mg/kg/day.** The LOAEL for inhibition of RBC AChE is 50 mg/kg/day. The NOAEL is 5 mg/kg/day**.**

The following table illustrates the ChE data from this study.

Dose	Plasma ChE		RBC AChE		Brain AChE	
	Males	Females	Males	Females	Males	Females
1 mg/kg/day	4.1 ns	32%*	0	8%ns	0	0
5 mg/kg/day	23%	35%*	1%ns	8%ns	0	18.1%*
50 mg/kg/day	64%*	64%*	39%*	32%*	28[a]	43%*

* Statistically significant $p < 0.05$. ns = not statistically significant. a. One animal.

Comments. The Original Data Evaluation Record (HED Document No.: 006940) established that, for serum cholinesterase inhibition the lowest dose tested (1 mg/kg /day) was the LOAEL in females but a NOAEL in males. For brain cholinesterase inhibition, the NOAEL was 1 mg/kg/day in both sexes. For red blood cell cholinesterase inhibition, the NOAEL was 5 mg/kg/day in both sexes.

At the October 5, 2000 meeting, the HIARC, following an in-depth review of the individual animal (cholinesterase) data determined that the 1 mg/kg/day is a NOAEL based on the following factors: 1) in spite of a 5-fold increase in the dose, the magnitude of response was unaltered between the 1 mg/kg/day (32%) and the 5 mg/kg/day (35%) doses (i.e., lack of a dose-response); 2) within animal analysis showed no differences in serum cholinesterase inhibition at 1 mg/kg/day when individual animal values at termination were compared to pre-treatment levels (-15% at 1 mg/kg/day and -18% at 5 mg/kg/day compared to -56% at 50 mg/kg/day); 3) statistical analysis of the data by HED staff indicated that only the 5 and 50 mg/kg/day dose groups were statistically significant by pair wise comparison; and 4) no brain cholinesterase inhibition was seen at 1 mg/kg/day compared to statistically significant and dose-dependent inhibition at the 5 mg/kg/day (18%) and 50 mg/kg/day (43%).

Dose and Endpoint Selected for Risk Assessment = NOAEL 1 mg/kg/day based on inhibition of serum (-35%) and brain (-18%) cholinesterase activity in females at 5 mg/kg/day.

Comments about Study/Endpoint: In general, dermal toxicity studies with thio-organophosphates conducted in rabbits tend to under estimate the toxicity of the chemicals because rabbits possess high concentrations of plasma carboxyl esterases which deactivate the chemical before it is converted into the active oxon. Diazinon is a thio-organophosphate which requires activation to the oxon in order to inhibit cholinesterase, and therefore, the 21-day dermal toxicity study in rabbits was not previously used for dermal risk assessments.

However, a closer re-examination of the results of the 21-day dermal toxicity study indicate that diazinon may be an exception to this hypothesis because: 1) adequate dermal absorption was demonstrated which in turn resulted in dermal toxicity (deaths in males at 100 mg/kg/day after 3 to 6 days of dosing); 2) the principal toxicological effect (serum and brain cholinesterase inhibition) was seen following repeated dermal exposure; and 3) comparable toxicity (mortality) was noted following oral (developmental toxicity study) and dermal exposures at the same dose (100 mg/kg/day, although different sexes were affected).

The dose of 1 mg/kg/day from the rabbit dermal toxicity study is obtained from a route-specific study. For these reasons, the HIARC determined that in the case of diazinon it is appropriate to use this study for dermal risk assessments. HIARC is aware that in the rat plasma ChE inhibition occurs at lower doses following an oral dose than was seen in the rabbit via dermal application. Therefore, the HIARC determined that a 90-day repeated dose dermal toxicity study in rats should be performed to verbify and refine this conclusion. The rat was selected since this species is considered to have dermal penetration properties closer to the human than the rabbit and a 90-day interval was chosen to allow for sufficient time for maximum inhibition of plasma and RBC cholinesterases.

5. Intermediate (7 Days to Several Months) and Long-Term (Several Month to Life-Time)

Study Selected: 21-day Dermal Toxicity - Rabbit

MRID No: 40660807

Dose and Endpoint Selected for Risk Assessment = NOAEL 1 mg/kg/day based on inhibition of serum (-35%) and brain (-18%) cholinesterase activity in females at 5 mg/kg/day.

Comments about Study/Endpoint: The HIARC determined that this study can also be used for these scenarios (intermediate and long-term) since the principal toxicity endpoint of concern (i.e., cholinesterase inhibition) was seen following dermal exposure. The HIARC, however, recommended that an additional 3x uncertainty factor (i.e., a Margin of Exposure of 300) be required for these scenarios. A MOE of 300 is required since the duration of treatment in the 21-day study may not be adequate to address the concern for achieving a steady-state following longer exposure. It was noted that in the 90 day oral studies in dogs, examination of the pattern of plasma ChE activity over time indicated that a steady state level of inhibition was reached by 90 days. This observation was supported by a similar examination of the blood cholinesterase data in the 1 year study in dogs which also contained a measurement time point at approximately 90 days.

4. Inhalation (Any Time Period)

Study selected: 21-day Inhalation Toxicity - Rat

MRID No. 40815002

Executive Summary (revised for this document): In a 21-day inhalation study, four groups of 15/sex Sprague-Dawley strain rats were dosed as control, 0.1, 1, 10 and 100 µg/L of diazinon MG-8 (87% purity) for six hour/day for a total of 21 or 22 consecutive days. No systemic toxicity was seen at any concentration. **The NOAEL for systemic toxicity is > 100 µg/L, the LOAEL is not established.**

The following table illustrates the plasma, RBC and brain cholinesterase inhibition data.

Concentration	Plasma ChE		RBC AChE		Brain AChE	
	Males	Females	Males	Females	Males	Females
0.1 µg/L	30%*9	56%*9	18%*9	NOAEL 4%9 ns	NOAEL 4%9 ns	NOAEL 6%9 ns
1.0 µg/L	50%*9	71%*9	53%*9	LOAEL 45%*9	LOAEL 13%*9	LOAEL 15%*9
10 µg/L	60%*9	76%*9	75%*9	75%*9	37%*9	44%*9
100 µg/L	80%*9	88%*9	91%*9	93%*9	62%*9	80%*9

* Statistically significant $p < 0.05$. ns = not statistically significant.

There is statistically significant inhibition for plasma ChE in both sexes and for RBC AChE for males at the lowest dose of 0.1 µg/L. **The LOAEL for plasma ChE in both sexes and for RBC AChE in *males* is < 0.1 µg/L; a NOAEL was not established for plasma ChE or RBC AChE inhibition. The LOAEL for RBC AChE in *females* and brain AChE in both sexes is 1 µg/L The NOAEL for RBC AChE in females and brain AChE in both sexes is 0.1 µg/L.**

Dose selected for Risk Assessment. LOAEL = 0.1 ppm based on statistically significant plasma cholinesterase inhibition in both sex and red blood cell cholinesterase inhibition in males. The converted dose = 0.026 mg/kg/day.

$$\frac{0.1 \text{ ppm} \times 10.26 \text{ L/hr (RV0} \times 6 \text{ hrs/day (duration)} \times 1 \text{ ppm/1000 mg (conversion)}}{0.236 \text{ kg (bw)}} = 0.026 \text{ mg/kg/day}$$

Comments about Study/Endpoint: This dose should be used for short, intermediate and long-term risk assessments. Since a NOAEL was not established for cholinesterase inhibition, an additional 3x factor is required (i.e., MOE = 300) for inhalation exposure risk assessments.

D. Margins Of Exposure for Occupational/Residential Exposures

A Margin of Exposure (MOE) of 100 is adequate for Short-Term dermal (occupational and residential) exposure risk assessments.

A MOE of 300 is required for Intermediate and Long-Term-term dermal (occupational and

residential) exposure risk assessments. The additional 3x uncertainty factor is required since the duration of treatment in the 21-day study may not be adequate to address the concern for achieving a steady-state following longer exposure.

A MOE of 300 is required for Short, Intermediate and Long-Term inhalation (occupational and residential) exposure risk assessments. The additional 3x uncertainty factor is required due to the use of a LOAEL (i.e., lack of a NOAEL in the critical study).

E. Recommendation for Aggregate Exposure Risk Assessments

For **acute** aggregate exposure risk assessment, the high end exposure values from food plus water should be compared to the acute RfD adjusted for the FQPA Safety Factor (i.e., the Population Adjusted Dose, PAD).

The **Aggregate Risk Index (ARI) should be used for Short, Intermediate and Chronic** aggregate risk assessments due to different MOEs for the short -term oral and dermal (MOE=100); Intermediate oral (MOE = 100); Intermediate and Long-Term dermal (MOE=300) and inhalation (MOE=300, for all 3 time period) routes.

For both **short- intermediate-and long-term exposures**, the aggregate systemic (oral), dermal and inhalation exposure risk assessments are appropriate due to the common toxicological endpoint (cholinesterase inhibition) seen via the three routes.

$$\text{Aggregate MOE}_{(\text{total})} = \frac{1}{\frac{1}{\text{MOE}_{(\text{oral})}} + \frac{1}{\text{MOE}\ (_{\text{dermal}})} + \frac{1}{\text{MOE}_{(\text{inhalation})}}}$$

III. CLASSIFICATION OF CARCINOGENIC POTENTIAL

In accordance with the Agency's Proposed Cancer Risk Assessment Guidelines of 1996, diazinon was classified as a **"not likely human carcinogen"** based on the lack of evidence of carcinogenicity in mice and rats. For details refer to the RfD/Peer Review Report dated June 6, 1997; HED Document No. 012412.

IV. FQPA ASSESSMENT

At the March 17, 1998 meeting the HIARC concluded that the prenatal developmental toxicity studies in rats and rabbits provided no indication of increased susceptibility of rats or rabbit fetuses to *in utero* exposure to diazinon. There was no indication of increased susceptibility in the fetuses as compared to parental animals in the two generation reproduction study. In the prenatal developmental studies no developmental toxicity was seen at the highest dose tested, and in the two-generation reproduction study, effects in the offspring were observed only at

treatment levels which resulted in evidence of parental toxicity. For details refer to the HIARC Report dated April 1, 1998, HED Document No. 012558 and the HIARC Report dated September 21, 1999, HED Document No 013745.

The FQPA Safety Factor Committee met on June 15 and 16, 1998 to evaluate the hazard and exposure data for diazinon and recommend application of the FQPA Safety Factor (as required by Food Quality Protection Act of August 3, 1996), to ensure the protection of infants and children from exposure to these pesticides. The FQPA Safety Factor Committee has determined that the 10x FQPA safety factor can be **removed** for diazinon**.** Refer to the FQPA Safety Committee Report dated August 6, 1998

IV. RECOMMENDATION OF ADDITIONAL STUDIES

90-day Dermal Toxicity Study in Rats: The HIARC determined that a 90-day dermal toxicity study in rats is required to assess the dermal toxicity potential of diazinon and to confirm the findings of the rabbit study. This study should be performed with both sexes and blood taken from all test groups, control and treatment, for cholinesterase (plasma and red blood cell) determination on Day -7, Day 0 (predose baseline), Day 1 (after dosing) and Weekly thereafter until termination at which time brain cholinesterase should be determined. Recovery group rats are recommended.

Developmental Neurotoxicity Study in Rats The Agency has recently issued a generic Data Call-In notice for a developmental neurotoxicity study for all chemicals in the organophosphate chemical class, including diazinon.

V. ACUTE TOXICITY

Acute Toxicity of Diazinon

Guideline No.	Study Type	MRID #(S).	Results	Toxicity Category
81-1	Acute Oral	41334607	LD_{50} = 882 (587-1326) mg/kg % = 968 (731-1283) mg/kg & =936 (742-1180) mg/kg combined	III
81-2	Acute Dermal	41334608	LD_{50} ~ 2000 mg/kg % (2/5 died) LD_{50} > 2000 mg/kg &	II
81-3	Acute Inhalation	41334609	4 hours exposure LC_{50} = 6.67 (0.189-242) mg/L % not determined for & = 9.36 (0.35-347) mg/L combined	III
81-4	Primary Eye Irritation	41334610	No corneal involvement. transient conjunctivae irritation (1 hr).	IV
81-5	Primary Skin Irritation	41334611	PIS = 0	IV
81-6	Dermal Sensitization	41334612 232008*	Not a sensitizer in guinea pig. Human study indicates 5-6/56 showed positive sensitization	NA
81-7	Delayed type neurotoxicity in hens	44132701	No evidence of delayed type neurotoxicity at 100 mg/kg.	NA

*Accession No.: MRID No.: not available.

VI. SUMMARY OF TOXICOLOGY ENDPOINT SELECTION

The doses and toxicological endpoints selected at the October 5, 200 HIARC meeting for various exposure scenarios are summarized below and should be used in risk assessments.

EXPOSURE SCENARIO	DOSE	ENDPOINT	STUDY
Acute Dietary	NOAEL=0.25 mg/kg	Plasma cholinesterase inhibition	Acute Neurotoxicity - Rat Special Study-Rat
	UF =100	**Acute RfD = 0.0025 mg/kg/day**	
Chronic Dietary	NOAEL=0.02 mg/kg/day	Consistent pattern of no adverse effects on cholinesterase inhibition.	4 week, 90 day and 1-year studies in dog 4 wee, 90 day and 2 -year studies in rat
	UF= 100	**Chronic RfD = 0.0002 mg/kg/day**	
Incidental Oral Ingestion -Short-Term	NOAEL= 0.25 mg/kg/day	Plasma cholinesterase inhibition.	Acute Neurotoxicity - Rat Special study -Rat
Incidental Oral Ingestion -Intermediate-Term	0.02 mg/kg/day	Consistent pattern of no adverse effects on cholinesterase inhibition.	90 day and 1-year Studies in dogs
Dermal Short-Term[a]	NOAEL= 1 mg/kg/day	Serum and brain cholinesterase inhibition.	21-day Dermal Toxicity -Rabbit
Dermal Intermediate-Term [b]	NOAEL= 1 mg/kg/day	Serum and brain cholinesterase inhibition.	21-day Dermal Toxicity -Rabbit
Dermal, Long-Term [b]	NOAEL= 1 mg/kg/day	Serum and brain cholinesterase inhibition.	21-day Dermal Toxicity -Rabbit
Inhalation (Any Time Period) [c]	LOAEL=0.1 : g/L (0.026 mg/kg/day)	Plasma cholinesterase inhibition in both sexes and RBC cholinesterase in males.	21-Day Inhalation - Rat

a = A MOE of 100 is adequate for Short-Term dermal occupational and residential exposure scenario.
b = A MOE of 300 is required for Intermediate and Long-Term dermal occupational and residential exposure scenarios
c = A MOE of 300 is required for Short, Intermediate and Long-term inhalation occupational and residential exposure scenarios